Subsurface topics
INEEL A Newsletter of Current Activities
SUBSURFACE —SCIENCE— INITIATIVE
July 2000 Vol.1 Issue 02

In This Issue

Initiative Director:
Dr. David L. Miller
(208) 526-9052 or BSH@inel.gov

Acting Science Director:
Dr. Bob Smith
(208) 526-9345 or RQS@inel.gov

For more information on INEEL research or to be added to the mailing list, use our online form at **http://subsurface.inel.gov/maillist.cfm** or email your name and address to:
Deborah Hill
526-4723 or DAHILL@inel.gov

Subsurface*topics* provides technical partners and interested researchers with information and updates about the INEEL's Subsurface Science Initiative and related research.

INEEL Hosts Meso-scale Subsurface Research Workshop

On May 8 and 9, 2000, the INEEL hosted a workshop to evaluate if and how meso-scale methods could be effectively used to link laboratory experimental data with the information needed to conduct field experiments. The INEEL is exploring research options to improve the understanding of subsurface contaminant fate and transport. At the request of DOE's Office of Environmental Management, the INEEL is seeking to enhance the connection between laboratory and field research. Workshop attendees included scientists from DOE headquarters, 6 DOE facilities, and 11 universities.

Meso-scale is defined as the physical and temporal experimental scales at which the coupling of chemical, biological, and physical processes occurring in the subsurface replicates the coupling that occurs under field conditions. The actual scale of a meso-scale experiment depends on the processes of interest and experimental objectives. Meso-scale research has great potential benefit to DOE because greater understanding of problems at the field scale can directly translate into cost savings and reduced uncertainty.

Presentations by Tom Stoops and Bob Starr of the INEEL established a background on DOE environmental issues and the need for broad-based, field-relevant research. Subsequent presentations illustrated how different disciplines have used meso-scale experiments to approach subsurface questions. Earl Mattson and George Redden of the INEEL, Steve Conrad of Sandia National Laboratories, Brian Wood of Pacific Northwest National Laboratories, Tim Ginn of the University of California-Davis, and Phil Jardine of Oak Ridge National Laboratory presented their research and discussed how meso-scale methodology is able to fill the information gap between laboratory and field experiments.

Meso-scale research

The workshop participants identified the situations and topics that could be addressed most effectively with meso-scale experiments, and the equipment and facility requirements that would be necessary to support them. The participants agreed that meso-scale methods are critical to understanding the complex nature of processes in the subsurface and should be used to:

- Accurately portray and observe coupled processes
- Observe increased spatial dimensionality
- Test scalability issues
- Experiment with hazardous and radioactive materials
- Conduct tests to determine field deployment abilities

INEEL Subsurface Science Initiative Acting Science Director, Dr. Bob Smith

Message from the Acting Science Director

One of the INEEL's major goals as a research institution is the advancement of knowledge for solving key Department of Energy problems. Traditional scales and approaches have become the limiting factor in achieving this goal. It is time to apply a new research paradigm using the focused application of meso-scale research to overcome these limitations.

Meso-scale research takes place at the physical and temporal experimental scales at which the coupling of chemical, biological, and physical processes occurring in the subsurface mimics the coupling that occurs under field conditions. Because the experiments are process focused, the actual physical scale for a meso-scale experiment depends on the processes of interest and experimental objectives.

The INEEL's new emphasis on meso-scale investigations provides a context for scaling up activities at the molecular and microbial community levels in the presence of other coupled processes. Applying the meso-scale experimental approach to subsurface problems will not only address scaling issues, but also contribute to one of this research institution's major goals – transforming experimental data into the advancement of knowledge.

- Maintain stricter control of research variables than is possible in the field
- Allow researchers to test hypotheses that are not possible at the field scale because of the cost of materials, labor, waste disposal, etc.

The workshop discussion emphasized that the optimal way to address these issues is to incorporate them into integrated sets of experiments, or "meso-scale campaigns," to promote collaboration among researchers in different disciplines.

Also discussed was the need for a new facility specifically focused on meso-scale subsurface science. It was recommended that a new facility be considered in context with existing facilities and not be redundant. The facility would also need to be flexible in design, having an ability to smoothly increase and decrease the scale and level of work to accommodate discipline differences and gradual increase in experiment size. The Initiative was encouraged to visit other meso-scale facilities to incorporate their experiences, ensure the success of joint arrangements, and encourage collaboration.

Contact: Dr. Bob Smith, RQS@inel.gov

INEEL Continues Recruiting for Subsurface Science Initiative

If you read *Science*, *Nature*, *ASM News*, *EOS*, or a handful of other scientific journals, you may have noticed the INEEL's Subsurface Science Initiative prominently featured in the recruiting section.

The INEEL continues to seek candidates for open positions, including the Initiative's Science Director and Discipline-Specific Technical Leads for chemistry, geosciences, physics, modeling, and biosciences. Positions are also open for senior and junior researchers, although most of these will be filled after the senior management and research team is in place.

Science Director

The Science Director's primary responsibility will be to expand the INEEL's current scientific capabilities into an internationally recognized location for conducting subsurface environmental science. The candidate should be a nationally recognized Ph.D. with an extensive track record developing and managing significant research programs related to subsurface science. Disciplines of interest include geology, geophysics, hydrogeology, vadose zone hydrology, geochemistry, and subsurface microbiology.

Experience in applying subsurface expertise in the area of environmental management would be valuable. The candidate should also have a distinguished record of scientific publication, demonstrated leadership and organizational skills indicating an aptitude for administration of an integrated research program, and an ability to facilitate research with organizations outside the INEEL.

Discipline-Specific Technical Leaders

Discipline-Specific Technical Leads will be hired as Science Fellows and will have wide latitude to develop research programs within the context of

INEEL Issues LDRD Calls for 2001 Proposals

The INEEL Applies Discretionary Funding to Subsurface Research.

In late May, the INEEL issued a call for research proposals for its 2001 Laboratory Directed Research and Development (LDRD) program. The INEEL's Subsurface Science Initiative was at the core of this call. The proposals received through this call are currently being evaluated.

LDRD funds are a discretionary resource used by National Laboratories to invest in long-term, high-risk, and potentially high-payoff research activities that extend the Lab's science and technology capabilities.

The INEEL's LDRD program supports its research mission in many ways. Because LDRD funds can be allocated within a relatively short timeframe, INEEL researchers can support the mission of the Department of Energy (DOE) and serve the needs of the nation by quickly responding to forefront scientific problems. The program enables the INEEL to attract and retain highly qualified scientists to carry out world-class research. In addition, the program supports new projects involving graduate students and postdoctoral fellows, contributing to the INEEL's education mission.

One of the primary goals of the LDRD program is to further advance and benefit the INEEL's science foundation. LDRD researchers are encouraged and financially assisted in publishing and presenting their work at conferences. Industry and university collaboration with the LDRD program is available with certain limitations.

The LDRD program relies on individual scientific investigators and the scientific leadership of the INEEL when allocating LDRD research dollars to achieve laboratory objectives. After submission, each proposal undergoes intense evaluation by both technical and managerial staff. Final decisions are made by a strategy council that determines the most balanced and beneficial projects. Projects selected in this year's LDRD awards are slated to begin October 2, 2000.

Research Areas

The LDRD program call for the Subsurface Science Initiative identified research needs pertaining to fluid-flow physics, biogeochemical transformations, and characterization. Subsets of these areas are:

Fluid flow physics

- Improving data collection techniques
- Parameter estimation
- Data interpolation
- Uncertainty analysis and quantification

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Biogeochemical transformations

- Environmental transformation rates
- Coupled biogeochemical/transport modeling
- Enhanced geomicrobiology characterizations
- Subsurface colloid behavior
- Co-contaminant interactions
- Evaluation and prediction of the long-term performance of caps and barriers for the stabilization of waste

Characterization

- Noninvasive measurement
- Point measurement
- Measurement integration

Recruiting

the overarching goals and vision of the program. Candidates must have a Ph.D.; an extensive peer-reviewed publication record in fundamental or applied research; demonstrated success in securing external funding through peer-reviewed competition; and experience managing large, collaborative programs. The candidates also must have demonstrated success in assembling and directing interdisciplinary research teams.

Many junior and senior research posts are being held open to allow the Initative's new leaders to select and fill the positions. To meet immediate needs, some positions for junior and senior researchers are currently posted in bioscience, geophysics, and geoscience modeling. Recruiting efforts will continue over the next several years.

Contact: Dr. Melinda Hamilton, HMN@inel.gov

Interested in Employment?

Subsurface Science Initiative job postings are on the Internet at http://www.inel.gov/resources/employment
Qualified candidates are invited to send their CV with a cover letter detailing their scientific, administrative, and leadership abilities to:

Subsurface Science Initiative Research Search Committee
c/o Melinda Hamilton
Idaho National Engineering and Environmental Laboratory
P.O. Box 1625
Idaho Falls, ID 83415-2203

Visiting Graduate Student Spurs INEEL Research

Abbie Aiken is Washington State University's only graduate student – so far this year – to move to Idaho for a summer of work in the INEEL's laboratories, but there will certainly be more in the future. Graduate interns are also likely to come from the other six universities of the Inland Northwest Research Alliance (INRA), a partner in the management of the INEEL.

Washington State University

"We absolutely hope to expand this and include more graduate students," said William A. Apel, Ph.D., microbiologist and Scientific Fellow at the INEEL, and affiliate faculty member in the Washington State University (WSU) Chemical Engineering Department.

"It's becoming a much broader collaboration than we've had in the past between the the INEEL and the INRA universities, and we'd like to make it even more seamless."

Apel heads the INEEL's Biologically-Based Catalysts for Processing and Detection in Harsh Service Conditions project, a program that supports and complements the Subsurface Science Initiative. Aiken's focus on nitrate reductase and its interactions with metals is only one part of the project. The project also includes studying the uses of other extremophilic microorganisms and their enzymes for purposes ranging from environmental monitoring to industrial catalysis.

"Placing university graduate students at INEEL allows us to access high-quality collaborators we might not otherwise have," Apel said. "Through the graduate students, you almost automatically have access to their advisors. It provides us [the INEEL] with a way to supplement our work force and gives us an active role in educating the next generation of environmental professionals, helping

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Enzymes a Key to Measuring Subsurface Pollution

Abbie Aiken has gone to extremes to develop a fast, portable, and inexpensive technology that can detect and measure heavy-metals contamination in groundwater. The extremes are bacteria, "extremophilic" bacteria that can survive at unusually high salinity and pH. Aiken, a chemical engineering doctoral candidate from Washington State University, is laying the scientific groundwork at the INEEL for a device that can use enzymes produced by extremophilic bacteria to characterize the metal content of groundwater samples at the wellhead.

"Otherwise, you have to take the samples to the lab and analyze them on expensive specialized machinery. That can take a while," she said. "A lot of these machines can cost more than your home and are not very portable."

As part of Aiken's doctoral thesis program, she is now serving her second summer internship at the INEEL under the National Science Foundation's Integrative Graduate Education and Research Training (IGERT) program in the Center for Multiphase Environmental Research at WSU.

WSU graduate students collected the Halomonas campisalis used in this research in the saline environment surrounding Soap Lake in central Washington State.

Aiken is concentrating on the nitrate reductase enzyme from one specific extremophilic bacteria, *Halomonas campisalis*. Her work began in summer 1999 doing activity assays on a commercial, assimilatory form of the enzyme. This assimilatory nitrate reductase catalyzes the reaction between nitrate and NADPH, a coenzyme important in production of sugar from carbon dioxide during photosynthesis. In solution, the reaction consumes the NADPH and produces nitrite. Nitrate reductase is sensitive to the presence of metals, however. Depending on the concentration of metal ions and the rate of the reaction, the resulting rate at which the NADPH concentration falls will vary. Beyond a certain threshold of metal concentration, the reaction stops completely. Aiken noted that both the rate at which NADPH concentration falls, and the threshold at which the reaction stops, also depend on which particular metal is present.

During her studies, Aiken measured the sensitivity of nitrate reductase to metals including lead, cadmium, zinc, nickel, chromium, and copper. NADPH absorbs strongly at an ultraviolet wavelength of 340 nanometers. Using a spectrophotometer to take readings every three seconds, she measured both the level of NADPH and the rate at which it reacts.

During her first summer of research, Aiken used NADPH as an indicator because it was a faster method of doing the preliminary characterization of the reactions than measuring nitrite concentration, which would have required additional reagents in reacting solutions. Nitrate reductase from the *Halomonas campisalis* will not use NADPH to facilitate the reduction of nitrate to nitrite. Instead, a different chemical, methyl viologen, is utilized as its electron donor.

Ultimately, however, Aiken said nitrite would be the indicator of choice in a working analytical field device. This summer, one focus of her work is to characterize the reactions for nitrite concentration.

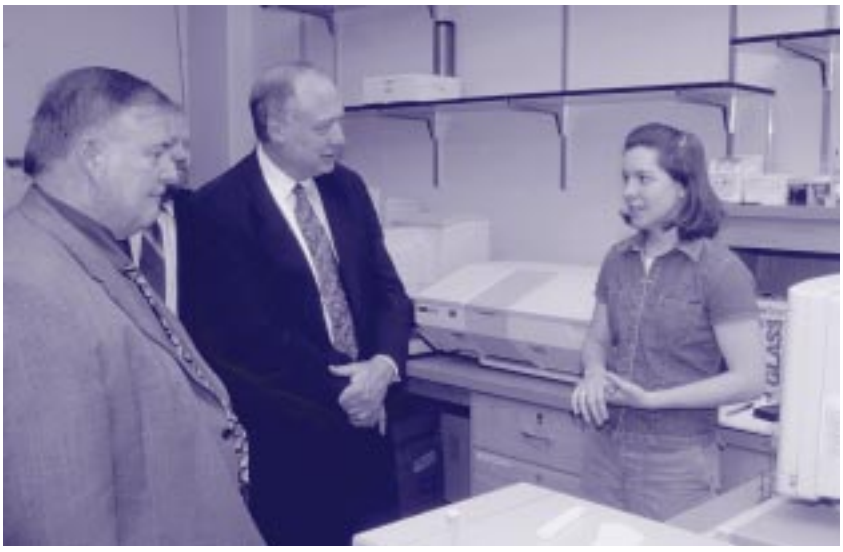
Another major focus of Aiken's work this summer is to characterize the nitrate reductase from *Halomonas campisalis* and to develop and refine procedures for purifying the enzyme and generating it in useful quantities. Currently, the commercial form of nitrate reductase comes from a "mesophilic" microorganism that lives in more moderate environmental conditions. The enzymes of extremophilic bacteria are believed to be more stable in the extreme conditions under which subsurface metal contaminants have to be measured. The exact form of a device, in which the enzyme assay of heavy metals content will be accomplished, has yet to be worked out.

There are obvious efficiency and cost benefits with the development of a portable device.

Aiken is also experimenting with metal chelators, chemicals that bind with specific metal ions. A chelator that binds a particular metal will make that metal unavailable for inhibiting the enzyme and affecting the reaction rate.

"Hypothetically, we could use metal chelators as a way of metals speciation," Aiken said. "Adding the right combination of metal chelators would wipe out the effects of all but one metal."

Contact: Dr. Bill Apel, WAA@inel.gov
Dr. James N. Petersen, jnp@wsu.edu or jnp@inra.org



Washington State University graduate student Abbie Aiken describes her research to INEEL and INRA managers.

Visiting Graduate

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them learn about the problems that are important to us."

Apel added that the program also serves as a recruitment mechanism. The INEEL gets to know the students and they get to know the INEEL. The relationship often leads to mutually beneficial permanent jobs.

According to Aiken, the internship gives students professional experience while they are still in school, and helps them visualize what they may be doing when they finish their degrees. Aiken confirmed that her exposure to scientists in multiple disciplines – from microbiology to chemistry – and the opportunities to discuss her work with them generates a lot of good ideas.

The mutual benefits resulting from the collaboration between the INEEL and the INRA universities are the reason outside internships are promoted by the WSU chemical engineering program and the National Science Foundation's Integrative Graduate Education and Research Traineeship, which supports a portion of Aiken's education.

"I'm living here and have an office here, so I get to meet all the people working on different aspects of the project," Aiken said. "I like that because it gives me a little more perspective on research related to my project, and I get to know the people personally."

For information about graduate internships at the INEEL, contact: Una Tyng, UNA@inel.gov

Chemically Assisted Composting Applied to TNT-Contaminated Soils

Before the contaminated soil is composted, it is mixed with acetone.

Using naturally occurring microbes to compost TNT-contaminated soil is a tried-and-true remediation method and an alternative to incineration. Unfortunately, the method doesn't work well with "chunks" of TNT, which aren't as easily broken down. Commonly used composting methods may leave this potentially carcinogenic substance in the soil.

INEEL microbiologists Corey Radtke, R. Michael Lehman, and Dr. Francisco F. Roberto searched for a way make the explosive chunks more accessible to the microbes. They looked at studies indicating that acetone could be used as a solvent to extract the TNT from the soil. However, previous researchers had been unable to find a method to safely recover the acetone containing dissolved TNT from the soil.

The INEEL group, which included Project Manager Dan Smith, tried composting the contaminated soil without removing the acetone. By the end of the process, the TNT was completely degraded and the acetone evaporated. The microbial populations at work degrading the explosive thrived under the modified conditions.

Variations in chunk size interfered with the researchers' ability to draw conclusions about how well the method worked. To reduce the variation, they sifted the soil with a mesh that removed TNT chunks over 2.4 millimeters. The sifted soil was held in compost reactors where the scientists tested how much acetone was needed, whether the presence of acetone slowed down the composting, and what effect acetone had on the microbial communities responsible for the TNT degradation.

Chunks of TNT and soil samples.

After determining the optimal concentration of acetone, the team sampled the composting reactions to see how fast the microbes were breaking down the TNT. Without acetone, the compost reactions had an average of 2,000 parts per million after 20 days. With the optimal concentrations of acetone added, the microbes degraded the TNT to less than 500 parts per million within one day, and TNT concentrations were down to safe levels within a week.

Treating the soil with acetone without composting did not degrade the TNT as fast as with composting, but how composting speeds up contaminant degradation is not yet known. The researchers suggest that the acetone may be dissolving the TNT, making it available to the microbial communities in the compost. Or, since acetone can be used as a food source by microorganisms, the solvent may be enabling the microbes to metabolize the TNT chunks in a way that isn't yet clear.

The researchers noted that the compost's temperature doesn't rise as fast with the addition of acetone, but temperature – usually an indication of microbial activity – does not seem to be important for TNT degradation. It was speculated that the acetone creates such a feast for the microorganisms that they don't have to work as hard to degrade the explosive substance.

To understand the effect of acetone on microbial communities, Lehman used a method that categorizes bacterial communities based on what they metabolize. Using a panel of 95 different bacterial foodstuffs, the INEEL team found that the compost reactors, with and without acetone, had different microbial communities at work.

"We're pretty sure there are at least three distinct communities doing the same thing," said Radtke. "The fact that there are so many gives us flexibility in the field. It makes the composting problem an engineering problem, not one where we have to worry about the science behind it."

For example, instead of having to grow certain bacterial strains in the laboratory to degrade TNT, users of this method will only need to ensure consistent soil conditions and recipes. The team has already scaled up its experiments to 30-gallon batches of soil at a time.

"The beauty of this method is that when the experiment is done," said INEEL microbiologist Corey Radtke, "the treated soil can go back into the ground and we never have to worry about it again."

Soil at old gunnery ranges and military wastewater lagoons is often contaminated with the explosive TNT. The INEEL has about a thousand cubic yards of TNT-contaminated soil, resulting from a World War II-era Naval gunnery range once located at the site. The particles of TNT range from about 56 grams to less than a hundredth of a gram. Although the TNT at the site is unlikely to pose an explosion hazard, the INEEL must investigate remediation alternatives, because the Environmental Protection Agency has identified TNT as a possible human carcinogen.

Users of this method will only need to ensure consistent soil conditions and recipes.

According to the Environmental Protection Agency, soils at more than 30 munitions test areas across the U.S. are contaminated with TNT. The site of the largest funded Unexploded Ordnance Remediation Project is a World War II Naval range on the Hawaiian island of Kaho'olawe.

Last November, Radtke and his colleagues presented their findings at a conference in Maui. A report on this work, by INEEL microbiologists, Corey Radtke, R. Michael Lehman, and Dr. Francisco F. Roberto, is published in an article titled "*Increased Biotransformation Efficiency of Chunk-TNT Contaminated Soil using Acetone Pretreatments*," Volume 4, Issue 1, pages 57-67 of *Bioremediation Journal*.

Contact: Corey Radtke, RADTCW@inel.gov
R. Michael Lehman, MIK4@inel.gov
Dr. Francisco F. Roberto, FFR@inel.gov

"The beauty ... is that when the experiment is done, the treated soil can go back into the ground and we never have to worry about it again."

A one cubic yard compost reactor holds the sifted soil ready for testing.

Initiative Notes

Subsurface Science Initiative Advisory Board to be established
An advisory board of leading scientists from universities, national laboratories, and government agencies is being identified to provide guidance to the Initiative. Advisory Board members will be announced this Fall.

INEEL/INRA delegation to visit the VEGAS facility
A delegation of scientists from the INEEL and INRA will visit an operating meso-scale facility (the University of Stuttgart's VEGAS facility) at the beginning of next fiscal year. The delegation hopes to learn from VEGAS scientists' experiences when designing the Subsurface Initiative's meso-scale campaigns.

Facility planning continues
INEEL planners continue to define future facility requirements to meet Subsurface Science Initiative and Environmental Management needs.

http://subsurface.inel.gov
Initiative Web Site To Be Launched

Subsurface.inel.gov, *set to launch in August, is geared to the research audience, and designed to expand with the Initiative.*

While the Internet was originally developed to foster collaboration among researchers, it has evolved into a much more far-reaching communication tool. The web site for the Subsurface Science Initiative is designed to meet two primary objectives: (1) deliver up-to-date information on Initiative research to the technical community, and (2) aid colleagues in accessing Initiative research in ways that are meaningful.

Initially, the site will provide project descriptions, publication references, biographical information on researchers, descriptions of research partners, and links to relevant research sites. Background on the Initiative will be provided. All site information will be searchable and sortable by problem area (e.g. vadose zone contamination), research area (e.g. biogeochemical transformations), specific discipline (e.g. actinide chemistry) or a combination of search criteria.

Because the site is constructed with a database, instead of being "hard-coded," content can be kept current, and the web of hyperlinks can be woven much more tightly to maintain greater utility to researchers. As the Initiative matures, site features will be customized to facilitate improved collaboration opportunities among researchers.

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INEEL Subsurface Science Initiative
Laboratory MS 2214
P.O. Box 1625
Idaho Falls, ID 83415-3911

Address Service Requested

http://subsurface.inel.gov